

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

Amend the paragraph on page 17, line 19 as follows:

Figure 3 shows comparison between the nucleotide sequence of the SCDH gene from the rice blast fungus registered with the gene bank (SEQ ID NO: 13), a nucleotide sequence (cDNA) of an SCDH gene from a standard strain (SEQ ID NO: 14) and a nucleotide sequence (cDNA) of an SCDH gene from a resistant strain (SEQ ID NO: 15).

Amend the paragraph on page 17, line 23 as follows:

Figure 4 shows comparison between the nucleotide sequence of the SCDH gene from the rice blast fungus registered with the gene bank (SEQ ID NO: 16), a nucleotide sequence (genome DNA) of an SCDH gene from a standard strain (SEQ ID NO: 17) and a nucleotide sequence (genome DNA) of an SCDH gene from a resistant strain (SEQ ID NO: 18).

Amend the paragraph on page 18, line 25 as follows:

Figure 7 is a schematic view showing a method for preparing plasmid Rice Blast wild SCDH cDNA and Rice Blast Mutant SCDH cDNA. Peptide sequence is SEQ ID NO: 19.

Amend the paragraph on page 22, line 11 as follows:

Next, the obtained total RNA was used to prepare cDNA containing a mutant SCDH gene from the resistant strain. In order to prepare cDNA containing the mutant SCDH gene, first, the obtained RNA (2 μ g) was mixed with 2 μ l oligo(dT)₂₀ (10 pmol/ μ l), 2 μ l each of Primer 1 (5'-GCAGTGATACCCACACCAAAG-3', 25 pmol/ μ l) (SEQ ID NO: 5) and Primer 2 (5'-TTATTTGTCGGCAAAGGTCTCC-3', 25 pmol/ μ l) (SEQ ID NO: 6) and RT-PCR beads (Amersham Biosciences) to a final volume of 50 μ l to prepare a reaction solution. The reaction took

place under the following conditions. For cDNA synthesis, reaction was performed at 42°C for 30 minutes, followed by reaction at 95°C for 30 minutes. Subsequently, for PCR reaction using the synthesized cDNA as a template, 35 cycles of 95°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute were repeated. After the final cycle at 72°C for 7 minutes, the reaction was carried out and terminated. The reaction solution obtained was purified after the reaction using the GFX PCR DNA and the Gel Band Purification Kit (Amersham Biosciences) to obtain the RT-PCR product. cDNA containing the SCDH gene from the standard strain and cDNA containing the mutant SCDH gene from the resistant strain B were also obtained in manners similar to the above-described method.

Amend the paragraph on page 23, line 3 as follows:

In addition, DNA containing the mutant SCDH gene from the resistant strain A was prepared using the obtained genome DNA. For preparing this DNA, first, 4 µl of the obtained genome DNA was mixed with 1 µl each of Primer 1 (5'-GCAGTGATACCCACACCAAAG-3', 25 pmol/µl) (SEQ ID NO: 5) and Primer 3 (5'-AGTTCGAACTGGAATTCAACCGGCACGCATGATGCATGCATTTA-3', 25 pmol/µl) (SEQ ID NO: 7) and PCR beads (Amersham Biosciences) to a final volume of 25 µl to prepare a reaction solution. The reaction took place under the following conditions. For PCR reaction using the genome DNA as a template, 40 cycles of 95°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes were repeated. After the final cycle at 72°C for 7 minutes, the reaction was carried out and terminated. The reaction solution obtained was purified after the reaction using the GFX PCR DNA and the Gel Band Purification Kit (Amersham Biosciences) to obtain the PCR product. DNA containing the SCDH gene from the standard strain and DNA containing the mutant SCDH gene from the resistant strain B were also obtained in manners similar to the above-described method.

Amend the paragraph on page 26, line 1 as follows:

Next, 50 µl TE buffer (pH 8.0) was added to the microtube, and the resultant was thoroughly agitated and centrifuged at 14,000 rpm for 10 minutes. The supernatant containing free genome DNA was transferred to another microtube and stored at -20°C. One to five µl of the supernatant was mixed with 1 µl each of Primer 4 (5'-ATGGGTTCGCAAGTTCAAAAG-3', 25 pmol/µl) (SEQ ID NO: 8), Primer 5 (5'-GTGGCCCTTCATGGTGACCTCCT-3', 25 pmol/µl) (SEQ ID NO: 9) and PCR beads (Amersham Biosciences) for a final volume of 25 µl to prepare a reaction solution. For PCR reaction, 40 cycles of 95°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes were repeated. After the final cycle at 72°C for 7 minutes, the reaction was carried out and terminated. The reaction solution was purified using the Invisorb Spin PCRapid Kit (Invitek) to obtain a PCR product. The PCR product contained in the reaction solution was subjected to sequencing reaction using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit from Applied Biosystems.

Amend the paragraph on page 26, line 15 as follows:

For the sequencing reaction, the PCR product as a template, 3.2 pmol of Primer 6 (5'-ACAAGCTCTGGGAGGCAATG-3') (SEQ ID NO: 10) and 8µl of terminator pre-mix were mixed to prepare a reaction solution for a total amount of 20 µl. For the sequencing reaction, 40 cycles of 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes were repeated. After the final cycle at 60°C for 7 minutes, the reaction was carried out and terminated. After the reaction, components such as the die terminator remaining in the reaction solution were removed by gel filtration using the Auto Seq G-50 (Amersham Bioscience). Then, the reaction product was subjected to sequence analysis using the ABI 310 Genetic Analyzer from Amersham Biosciences. By using a 47 cm x 50 µm short capillary column from Amersham Biosciences, mutation of the amino acid valine at position 75 into methionine was confirmed in a short time of about 35 minutes per sample.

Amend the paragraph on page 27, line 8 as follows:

As in Example 3, a genome DNA solution was simply prepared by irradiating rice blast fungus filamentous mycelium with microwaves. Five μ l of this genome DNA solution were mixed with 1 μ l each of Primer 6 (5'-ACAAGCTCTGGGAGGCAATG-3', 25 pmol/ μ l) (SEQ ID NO: 10), Primer 5 (5'-GTGGCCCTTCATGGTGACCTCCT-3', 25 pmol/ μ l) (SEQ ID NO: 9) and PCR bead (Amersham Biosciences) for a final volume of 25 μ l to prepare a reaction solution. For PCR reaction, 40 cycles of 95°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes were repeated. After the final cycle, the reaction was terminated at 72°C for 7 minutes. As a result of this reaction, 215 bp PCR product was obtained. The components such as taq DNA polymerase and primers remaining in the reaction solution were removed using GFX PCR DNA and the Gel Band Purification Kit (Amersham Biosciences).

Amend the paragraph on page 28, line 18 as follows:

In order to incorporate a scytalone dehydratase gene from a rice blast fungus into a protein expression vector pGEX-2T (Amersham Biosciences), RT-PCR was conducted using Primer 7 (5'-ATCGTCGACGTGAATTCGTCTTGTAAGCCGCCAAC-3') (SEQ ID NO: 11) and Primer 3 (5'-AGTTCGAACTGGAATTCAACCGGCACGCATGATGCATGCATTTA-3') (SEQ ID NO: 7) having *Eco*RI cleavage sites at their terminals. The RT-PCR was conducted according to the method described in Example 1. Primers 7 and 3 were located upstream and downstream from the open reading frame (ORF) of the SCDH gene, respectively, so as to flank the whole coding region for the SCDH enzyme.

Amend the paragraph on page 32, line 4 as follows:

A simple assay for Val75Met mutation in an SCDH enzyme from a rice blast fungus was considered by applying the PCR-RFLP method. Similar to Example 3, a rice blast fungus filamentous mycelium was irradiated with microwaves to simply prepare a genome DNA solution.

Five μ l of this genome DNA solution was mixed with 1 μ l each of Primer 8 (SEQ ID NO: 12, 5'-TTCGTCGGCATGGTCTCGAGCATCTAG-3', 25 pmol/ μ l), Primer 5 (5'-GTGGCCCTTCATGGTGACCTCCT-3', 25 pmol/ μ l) (SEQ ID NO: 9) and PCR bead (Amersham Bioscience) for a final volume of 25 μ l to prepare a reaction solution.